

MicroRNA Signatures of Colonic Polyps on Screening and Histology

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Abstract

Colorectal cancer and adenoma adjacent to cancer exhibit distinct microRNA (miRNA) alterations in an apparent mucosa-to-adenocarcinoma sequence. The pattern of microRNAs in screen-detected polyps in relation to histologic features and cancer risk has not been investigated. miRNA expression analysis was performed on normal mucosa (NM), hyperplastic polyps (HP), tubular adenomas (TA), tubulovillous adenomas or high-grade dysplasia (TVHG), and serrated polyps [sessile serrated adenoma/polyps (SSA/P) and traditional serrated adenomas (TSA)] in biopsy specimens from 109 patients undergoing screening/surveillance colonoscopy. Generalized linear models were used to identify differentially expressed miRNAs by histologic type and logistic regression to identify miRNA predictors of histopathology. False discovery rate (FDR) was used to control for multiple comparisons. We identified 99 miRNAs differing in at least one of five histopathologic groups (FDR ≤ 0.05). In a comparison of

HPNM versus TVHG, the top most upregulated and down-regulated miRNAs in HPNM included miR-145, -143, -107, -194, and -26a (upregulated), and miR-663, -1268, -320b, -1275, and -320b (downregulated; FDR $P < 0.05$). miR-145 and -619 showed high accuracy to discriminate low- from high-risk polyps without serrated histology (TVHG vs. HPNM + TA; CI, 95.6%), whereas miR-124, -143, and -30a showed high accuracy of separating high-risk polyps (TVHG + TSA) from low-risk polyps (HPNM + TA + SSA/P; CI, 96.0%). For TSAs, miR-125b and -199a were uniquely downregulated relative to HPNMs, and miR-335, -222, and -214 discriminated between non-serrated and serrated histology. Our data support the presence of colorectal cancer-associated miRNA alterations in screen-detected adenomas that may be useful for risk stratification for surveillance interval planning. *Cancer Prev Res*; 9(12); 942–9. ©2016 AACR.

Introduction

The conventional adenoma-to-carcinoma and serrated-to-carcinoma progression models involve distinct molecular events involved in deregulated growth and tumorigenesis (1). These include altered expression of small noncoding microRNAs (miRNAs) that bind to and destabilize/sequester target mRNA (2, 3). As such, a number of miRNAs have been reported to be altered in colorectal cancers, a handful of which are highly reproducible (4). Specifically, miR-106a has been reported to be consistently upregulated in colorectal cancers

where miR-30a-3p, -139, -145, -125a, and -133a are consistently downregulated (4).

While limited to a handful of studies, a few specific miRNAs have been implicated in the development of adenomatous polyps, though their importance in the transition from benign polyp to invasive carcinoma is largely unknown (5–9). Nagel and colleagues reported overexpression of miR-135a and -135b in colonic adenoma, with functional studies supporting their role as oncogenic miRNAs acting as inhibitors of adenomatous polyposis gene (APC) translation (5). Subsequently, Oberg and colleagues partly corroborated these findings reporting overexpression of miR-135b in adenomas (9). In contrast, the latter group reported decreased miR-135a in adenomas, an important difference between the two studies, given that MYC is a target of miR-135a and loss would release miRNA repression of MYC transcripts (10). To gain insight on miRNA patterns in adenoma associated with carcinoma, Bartley and colleagues profiled miRNA patterns in normal mucosa and foci of low- and high-grade adenoma adjacent to microsatellite-stable (MSS) colorectal cancers in a patient series (8). Two major patterns of miRNA alterations in adenoma development were identified: one pattern distinguished between normal mucosa and low-grade adenoma (i.e., "early pattern"), and one distinguished between normal mucosa and high-grade adenoma (i.e., "late pattern"). Changes between normal mucosa and low-grade adenoma miRNAs were consistent with Wnt pathway activation, whereas changes in high-grade adenoma included 33 miRNA alterations not present in low-grade foci. These included downregulation of the miR-143/145

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cluster and miR-125 noted above to be reproducibly downregulated in colorectal cancer (4). In another study of adenoma (11), downregulation of miR-143/145 was noted in ~65% of adenomas studied, supporting the loss of this oncomiRNA during adenoma development. This finding is important, given the role of the miR-143/145 cluster in regulating oncogenic KRAS, for which gain-of-function point mutations are common in adenomatous polyps (12).

With miRNA expression profiling offering new insights on tumorigenesis and the need to better understand and identify differences between benign adenoma and more clinically aggressive adenoma for patient risk stratification, we assessed miRNA patterns in screen-detected polyps from cancer-free patients undergoing screening colonoscopy across major histologic types. Here, we show that changes in miRNAs previously reported as deregulated in colorectal cancers and those in late adenoma adjacent to colorectal cancer accurately discriminate between low- and high-risk histologic polyp types in cancer-free patients undergoing screening.

Materials and Methods

Patient and sample selection

We examined colonic adenomas that were collected from patients undergoing screening colonoscopy during the years of 1990–2000, and from patients who were participants of the University of Arizona Wheat Bran Fiber Trial (WBF Trial; ref. 13) and the Ursodeoxycholic Acid Trial (UDCA Trial; ref. 14). These were two large polyp prevention studies for the further prevention of metachronous colorectal adenomas. All samples examined in our study were baseline samples from consented subjects participating in those trials. Subjects were therefore not on any drugs and were not following or adhering to any dietary modifications. We excluded all patients with a familial colorectal cancer syndrome or evidence of hyperplastic polyposis.

Patient information queried from the WBF and UDCA database included: age, gender, family history of colorectal cancer, location of lesion, history of smoking, and histology of the adenoma. We examined a total of 123 samples from 109 patients for miRNA expression. Briefly, from a total sample set of 109 unique patients, 96 provided one polyp, with an additional eight patients providing two samples of the same polyp. One patient provided three samples of the same polyp, and four patients provided two samples each of a different polyp. This specimen set allowed us to conduct a differential expression analysis of histology on 113 unique colonic polyps and to assess intra- and interpolyp variability in 27 samples from 13 patients.

The WBF and UDCA trials were approved by the University of Arizona Human Subjects Committee and local hospital committees, and written informed consent was obtained from each participant prior to study enrollment. The IRB approval obtained for our study is part of the initial approval that had included permission to review any colonoscopy sample at a later date.

Histologic classification of polyp type

All tissue samples were formalin-fixed, paraffin-embedded (FFPE) specimens obtained as part of routine pathology for clinical care. Normal colonic mucosa was obtained distant to the adenoma at the time of biopsy. Polyps were evaluated and categorized by three GI study pathologists (S.R. Hamilton, A.N. Bartley, and A.K. Bhattacharyya) on histologic type and subse-

Table 1. Specimen and patient characteristics included in analyses of miRNAs

Age (mean, SD)	66.9 years, ±9.2
Male, <i>n</i> (%)	73 (65%)
Histology, <i>n</i> (%)	
HPNM	23 (20%)
SSA/P	13 (12%)
TA	26 (23%)
TSA	11 (10%)
TVHG	40 (35%)
Positive family history of colorectal cancer	17 (15%)
Distal (left-sided) anatomic location	74 (65%)
Current smoker	15 (13%)

quently grouped for analyses as normal mucosa (NM), hyperplastic polyp (HP), tubular adenoma (TA), sessile serrated adenoma polyp (SSA/P), traditional serrated adenoma (TSA), and any tubulovillous or villous adenoma with high-grade dysplasia (TVHG). Samples were grouped, based upon histology and malignant potential into five groups: HPNM [lowest], SSA [low], TA [low], TSA [high], and TVHG [highest] (15, 16). This classification schema was selected based on the level of subsequent cancer risk, as defined in the Guidelines for Colonoscopy Surveillance after Screening and Polypectomy: A Consensus Update by the US Multi-Society Task Force on Colorectal Cancer (17). No SSA/Ps with cytologic dysplasia were present in the study set. The subject population studied and the polyps examined are summarized in Table 1 by group. Sixty-five percent of the population were male (65%), had a mean age of 66.9 ± 9.2 years, and were more likely to have distal colon or rectal polyp (17, 18).

MicroRNA analysis

For miRNA isolation from tissues, a demarcated hematoxylin and eosin-stained slide was used to guide macrodissection of regions of interest containing the defined predominant histologic feature(s) on which the tissue was classified. Total RNA, including miRNA, was isolated from the region with use of the miRNeasy FFPE kit (Catalog #217504; Qiagen), per manufacturer's recommended protocol. Total RNA yield from microdissection on the RT-PCR validation set only from ten × 5-μm sections/each (mean = 7.24 μg, median = 3.69 μg).

Microarray assays were performed by ALMAC Diagnostics. Total input RNA in the array assay was 500 ng. Total RNA, including miRNA, was labeled and hybridized to the GeneChip-miRNA v. 1.0 microarray (Affymetrix). There is a total of 825 human miRNA probe sets on this array. The sample processing occurred in a single batch and arrays were run on two different days as 52 (46%) and 61 (54%) specimens for in hybridization groups 1 and 2, respectively.

Technical validation of the array results was performed on a set of 24 samples randomly selected from the original sample. Included in the validation were seven HPNM, three TAs, three TSAs, and eleven TVHGs. Complementary DNA (cDNA) was made from total RNA, using a qSTAR First Strand cDNA synthesis kit (Origene), per manufacturer's protocol. SYBR Green qPCR was then performed, utilizing the three miRNA primer pairs of interest: hsu-mir-143, GGUGCAGUCGUGCAUCUCUGGU, MIMAT0004599; Hsu-mir-145, GUCCAGUUUCCAGGAAUCCCU, MIMAT0000437, and Hsu-mir-93, CAAAGUGCUGUUCGUGCAGGUAG, MIMAT0000093 (Origene), per manufacturer's protocol. Those three miRNAs were chosen from the list of 99 miRNAs that were differentially expressed in at least one histology

group. Validation assays were performed in triplicate on an Applied Biosystems 7900HT real-time PCR system (Life Technologies). miRNA expression was normalized to 5S. The PCR efficiencies between the target(s) and the 5S endogenous control were relatively equivalent. miRNA validation expression analyses were determined, using the comparative C_t (threshold cycle) method to calculate the relative expression level, using the equation: $\log_{10}(2 - \Delta C_t)$, where $\Delta C_t = (CT_{miR} - CTRN5S)$.

Data processing and quality control

An overview of the data processing, quality control, and analysis workflow is provided in Supplementary Fig. S1. Individual array data (.cel files) were preprocessed and normalized, using the robust multichip analysis (RMA) routine (18), available in the Partek Genomics Suite 6.6. The following options were specified: RMA background adjustment, PM values only, quantile normalization, \log_2 transformation, and median polish summarization. The complete normalized data were filtered to include only human probe sets, and probe sets identified as "dead miRNAs" in the miRBASE database were excluded. This resulted in 825 human probe sets for the statistical analysis.

Data visualization tools (e.g., box plot, hierarchical clustering, and multidimensional scaling) were used to assess general data quality and identify any underperforming or outlier samples to reduce the impact of non-biological factors influencing statistical testing results. The box plot in Supplementary Fig. S2 indicates multiple probe sets with very high signal intensities. We identified 16 miRNA probe sets with consistently high expression (defined as the expression level > 4 over 90% of all samples), and investigated further through miRBASE. These probe sets were identified as being associated with colorectal cancer, other cancers, and the colorectal microRNAome (Supplementary Table S1).

Inter- and intrapolyp correlation

In nine subjects, more than one block was available for the same polyp. Eight subjects had the same polyp in two blocks, and one subject had the same polyp in three blocks. The correlation of miRNA expression levels within the same polyp (intra-polyp) was available for 19 samples (Supplementary Table S2). An additional four individuals presented with two unique polyps of the same histologic type. For these, we analyzed the interpolyp correlation. Variance component analyses were performed for these samples, and intraclass and interclass correlations were high. The intraclass correlation coefficient (ICC) was 0.97 and 0.47 for intra- and interpolyps, respectively. Based on the ICC, we subsequently considered intrapolyp as one event, randomly selecting one sample from the same polyp. For participants with more than one unique polyp, each one was treated as a separate sample to assess miRNA pattern by histologic type. A scatterplot of the mean difference against the mean average of the pairs is shown in Supplementary Fig. S3.

Statistical analysis

To identify significantly differentially expressed miRNAs, we fit a generalized linear model with histology and scan date as two main factors for each miRNA. Benjamini and Hochberg false discovery rate (FDR) method (19) was used to correct for multiple testing. miRNAs with the histology FDR $P < 0.05$ were considered differentially expressed in at least one histologic group. Further, pairwise comparisons were performed among all histologic categories (HPNM, TVHG, TA, TSA, SSA/P), and a comparison of

high-risk (TVHG and TSA) versus low-risk (TA and SSA) groups, using the contrast statements was also performed. For each comparison, we applied the Benjamini and Hochberg FDR method (19) to correct for multiple testing. We considered miRNAs with FDR $P < 0.05$ and the fold change > 1.5 (upregulated) or < -1.5 (downregulated) as differentially expressed miRNAs.

Ninety-nine probe sets with a significant main histology effect (FDR $P < 0.05$) were selected for further graphical evaluation of their molecular signature, using a heat map and hierarchical clustering. In addition, we performed a canonical discriminant analysis and graphically examined separation of histology groups, based on the first three canonical variables. The canonical discriminant analysis is a dimension-reduction technique similar to principal component analysis (PCA), and attempts to identify linear combinations of the miRNA \log_2 signals that provide maximal separation among histologic groups (20). Clinical factors associated with more advanced histology were considered, using a logistic regression model and stepwise procedure, to identify miRNAs and patient characteristics (age group, sex, family history, smoking history, and adenoma location) predictive of high malignant potential (TVHG) compared with low malignant potential (HPNM + TA). Only the miRNA signature remained significant and is shown in the results.

The accuracy of the final model was represented as concordance index (c-index), a measure of percentage correctly specified for the model building data set, while the accuracy of the model selection procedure was represented as the 5-fold cross-validation area under the curve (AUC). Because of the small sample size, all of the data were used for model building, and a 5-fold cross-validation procedure was used to assess the accuracy of the model selection procedure.

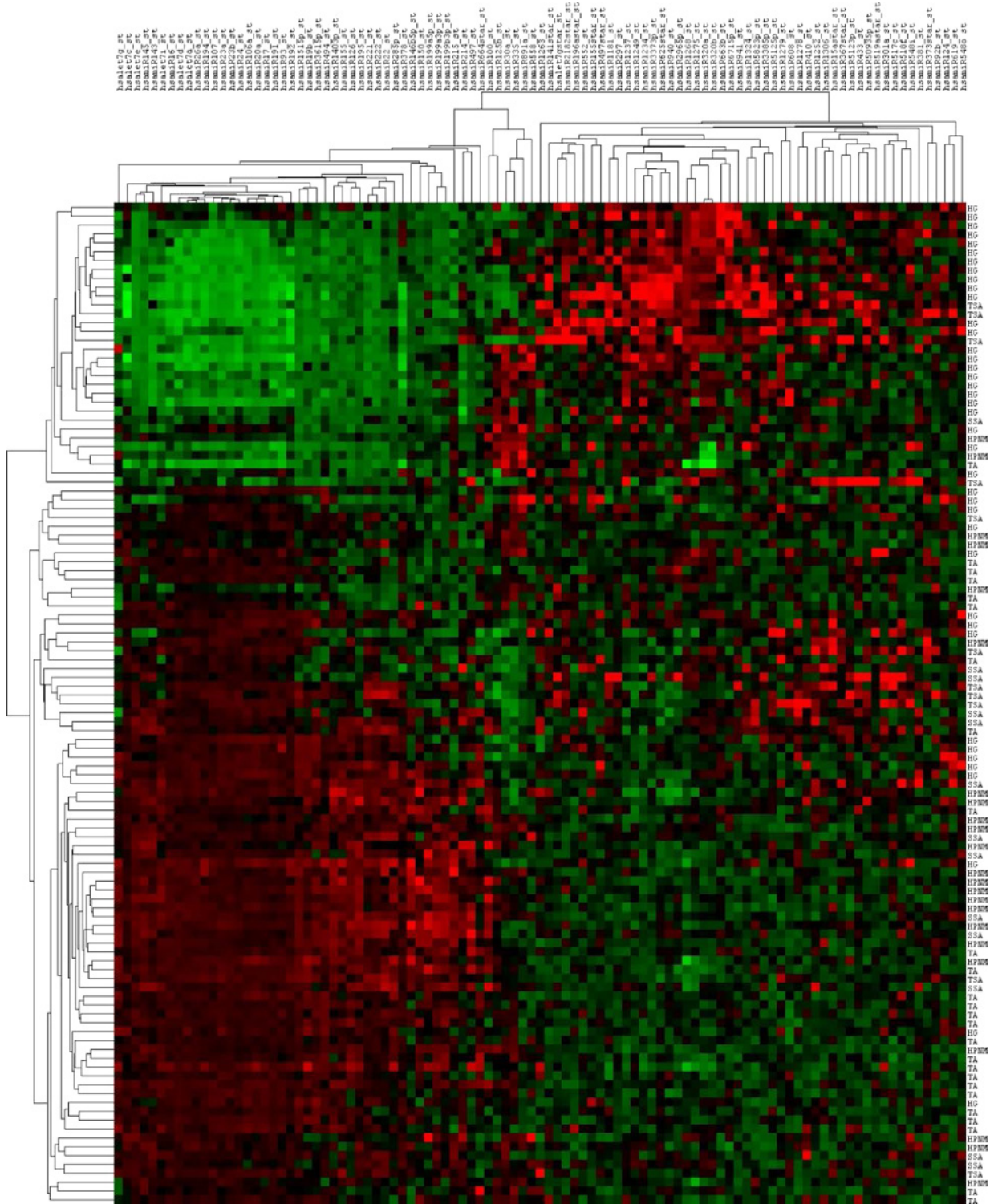
Exploratory graphical analyses for inter- and intrapolyp variability were conducted, using hierarchical clustering, multidimensional scaling, matrix plots, and box plots. Variance component analysis and general lineal models were conducted to assess probe-specific, as well as overall intra- and interpolyp variations.

Results

miRNA profile discriminates polyp histology

A heat map of probe intensity by histologic type is shown in Fig. 1. This heat map shows the clustering of the adenomas by their histology (i.e., the HPNM or TVHG), based on their associated miRNA signatures. A total of 99 miRNAs (Supplementary Table S3) were found to differ significantly in their expressed levels across the five histology types (e.g., mean signal intensities were significantly different in at least one histology group at FDR $P < 0.05$). A canonical discriminant analysis, including the 99 miRNAs (Fig. 2), showed clear separation of the five histologic groups from each other. We conducted 10 pairwise comparisons of one histology group versus another. The most significant differences in pairwise analysis (Table 2) occurred between TVHG and other histology groups. In particular, when TVHG was compared with HPNM, 47 miRNAs were differentially expressed. When TVHG was compared with SSA/P, 25 miRNAs were found to be differentially expressed; when compared with TAs, 45 miRNAs were differentially expressed.

The five miRNAs (higher or lower) with the greatest fold differences between TVHG and the HPNM are shown in Table 3. miR-145, -143, -107a, -194, and -26a were highly expressed in HPNM relative to TVHG, whereas miR-663b, -1268, -320a, -320b,



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Figure 1. Hierarchical clustering of differentially expressed miRNAs by histologic type. Clustering was based on the profiling of 99 miRNA probes (FDR < 0.05). Probe intensity is represented in the columns and the histologic type are presented in the rows. Green indicates highly expressed probes while red indicates probes with low expression. Histologic types are annotated as HPNM, TA, SSA/P, TSA, any tubulovillous or villous adenoma TV), and high-grade dysplasia (HG).

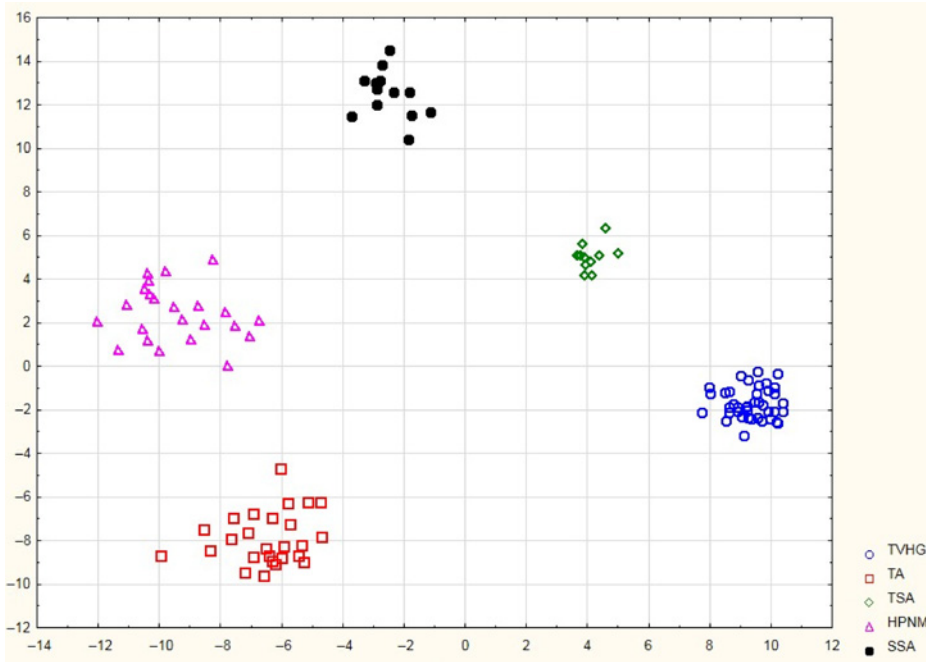


Figure 2. Scatterplot illustrating canonical discriminant analysis findings for 99 miRNAs across five histologic groups. Histologic types are annotated as HPNM, TA, SSA/P, TSA, and any TVHG.

and -1275 were highly expressed in TVHG relative to HPNM. Using binary logistic regression, we found that low expression of either of the two highly correlated miRNAs (-145 or -143 [$r = 0.87$]) accurately separated TVHG from HPNM (c -index 0.938). miR-143 and -145 function as an anti-oncomiRNA cluster (21) which, as noted, is reproducibly downregulated in colorectal cancers (4).

miRNA expression pattern as classifier of polyp type

To assess the predictive performance of the miRNA expression-based classifier for high-risk adenoma in the context of serrated histology, we conducted a binary logistic regression of high-risk lesions (TVHG + TSA) versus low-risk lesions (HPNM + TA + SSA/P). In this analysis, miR-143, -124, and -30a were identified as independent predictors of high-risk region (c -index = 0.960). Like the miR-143/145 oncomiRNA, miR-30a is reproducibly

downregulated in colorectal cancers (10). Among its functions, miR-30a inhibits transforming growth factor- β induction of Snail-mediated epithelial-to-mesenchymal transition (EMT) by direct binding to the 3'-UTR of Snail (22), with evidence that miR-30a acts to negatively regulate EMT, where downregulation is associated with invasion and metastasis (23).

miRNA expression in serrated polyps

No previous studies of miRNA expression have considered serrated histology. Histologically and definitively, all conventional adenomas show epithelial dysplasia, whereas SSA/Ps sometimes display cytological atypia or dysplasia without the classic dysplastic features (24). SSA/Ps are considered the precursor lesion for many sporadic colorectal cancers with microsatellite instability and for other colorectal cancers characterized by hypermethylation (25–27). Initially, we evaluated TSAs and SSA/Ps as

Table 2. Number of significantly differentially expressed miRNAs in pairwise comparison of histologic types at FDR < 0.05 and fold change ≥ 1.5 and ≤ -1.5

	TVHG vs. TA	HPNM vs. TVHG	TVHG vs. SSA/P	HPNM vs. TSA	TSA vs. TA	HPNM vs. SSA/P	HPNM vs. TA	TVHG vs. TSA	SSA/P vs. TSA	SSA/P vs. TA	Frequency
											763
											18
											14
											11
											4
											4
											3
											3
											2
											2
											1
											1
											1
											1
Number of DE miRNAs	49	46	26	16	5	0	0	0	0	0	

NOTE: A dark shaded entry represents the presence of differential expressed (DE) miRNAs for a specified comparison. For example, there are 18 miRNAs that are differentially expressed between TVHG vs. TA, HPNM vs. TVHG, as well as TVHG vs. SSA/P.

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Table 3. Top five miRNAs with higher and lower expression in HPNM compared with TVHG by the fold change among those with FDR $P < 0.05$

miRNA	P	Fold change
hsa-miR-107_st	7.51E-05	4.6
hsa-miR-143_st	7.24E-08	5.2
hsa-miR-194_st	5.70E-05	6.6
hsa-miR-26a_st	5.20E-06	6.7
hsa-miR-145_st	7.76E-11	7.7
hsa-miR-663b_st	0.0003	-2.1
hsa-miR-1268_st	0.001	-2.0
hsa-miR-320a_st	0.001	-1.7
hsa-miR-320b_st	0.009	-1.7
hsa-miR-1275_st	0.015	-1.6

separate groups, given a presumed distinct etiology (28). While we did not identify significant differences in miRNAs between TSAs and HGTVs, there were several differentially expressed miRNAs observed between HPNMs and TSAs. Notably, no significant differences in miRNAs between SSAs and TSAs (Table 2) were identified, though we were restricted by small sample size in each of these groups. In Table 4, we show the miRNAs with greatest differences in expressed levels between TSAs and HPNM.

In an attempt to tease out serrated specific miRNAs, we explored pairwise comparisons that emphasized the presence of serrated histology to increase our sample size. Specifically, we compared miRNA expression patterns between TVHG and TAs to a group with any serrated feature (SSA/P and TSA). We found miR-335 was significantly overexpressed by 2-fold (FDR $P = 0.021$) in non-serrated tissues, when compared with serrated lesions. Additionally, miR-222 and -214 were significantly downregulated by 2.35-fold (FDR $P = 0.02$) and 1.51-fold (FDR $P = 0.03$), respectively, in serrated polyps.

miRNA expression differences between traditional and serrated adenomatogenesis

To further explore the hypothesis that traditional and serrated type adenoma derive from discrete etiologies, we conducted an ordinal logistic regression with the assumption that TVHG develop as a linear progression of HPNM to TA, and that TSA develop from HPNM to SSA. Assuming linear progression, miR-145, miR-30a, miR-517c, and miR-619 were found to be independent predictors of progression within a traditional pathway with a concordance index of 93.5% for the HPNM to TVHG. For the serrated pathway, miR-125b and miR-320a emerged as independent predictors of progression within a presumptive serrated pathway with concordance index of 84.7%.

Discussion

Here, we observed that that miR-143/145 and -30a accurately more advanced adenomatous polyps from lower-risk lesions,

Table 4. Top five miRNAs with higher expression in HPNM compared with TSA^a by fold change among those with FDR $P < 0.05$.

miRNA	P	Fold change
hsa-miR-145	0.02	3.6
hsa-miR-30a	0.02	3.1
hsa-let-7f	0.04	2.9
hsa-miR-195	0.004	2.9
hsa-miR-335	0.02	2.8

^aNone of miRNAs were significantly downregulated in HPNM when compared with TSA. Significant downregulation is defined as FDR $P \leq 0.05$ and fold change < -1.5 .

including the common small tubular adenomas detected during colorectal cancer screening of cancer-free individuals. Despite the small number of samples in each histologic group, these findings are significant as they demonstrate that miR-143/145 and -30a, which are consistently downregulated in colorectal cancer (4) and decreased in foci of high-grade dysplasia adjacent to invasive disease (8), are also altered in more advanced adenoma of cancer-free individuals undergoing screening.

Interestingly, we found that TSAs and HGTVs, both high-risk adenoma, share miRNA expression features with each other, as well as with colorectal cancer and high-grade adenoma-adjacent invasive disease. In particular, TSAs exhibited dramatic downregulation of miR-143/145. Unlike tubular adenomas, TSAs make up <1% of all colonic adenomas, but frequently occur with an existing intramucosal carcinoma component (10%–30%). It is estimated that as many as 30% of all colorectal cancers develop from these rare but aggressive precursors (29, 30). As such, the loss of miR-143/145 and -30a in adenomatous polyps may be informative for identifying high-risk adenomatous polyps at a molecular level independent of histologic features.

When we examined the expression of miR-143,-145, and -30a among "low-risk" lesions and compared it with the median expression in the "high-risk" group, we found that 3% of miR-143, 0% of miR-145, and 23% of miR-30a expression below the median expression of the "high-risk group," indicating that these rather benign polyps may harbor molecular features of high-risk premalignant lesions (Supplementary Fig. S3).

Chen and colleagues were the first to demonstrate that miR-143 directly inhibits KRAS gene expression, with work by Akao and colleagues (11) demonstrating that miR-143/145 is decreased in ~65% of adenomatous polyps (31). These results have been argued to demonstrate that reduced miR-143/145 expression is an important molecular event in adenomatogenesis. We did not observe significant differences in miR-143 or -145 expressions between screen-detected, small tubular adenoma and normal mucosa/hyperplasia. This result suggests an important difference between early and late adenoma and raises the possibility that loss of miR-143/145 is necessary for progression of early to late adenoma and invasive carcinoma and is perhaps a useful biomarker for discriminating low- and high-risk adenoma.

Unlike the previous study of low-grade foci from cancer patients (8), we did not observe significant differences in miRNAs between HPNM and TA in our non-cancer patient population (Table 2). While limited by sample size, this suggests that main effects of specific miRNA alterations in adenoma to carcinoma are absent in the small TAs. In contrast, we did find evidence for a "late" miRNA expression pattern that separated more advanced TVHG from normal mucosa and hyperplastic polyps. Specifically, 47 miRNAs differed between HPNM and TVHG, of which the majority overlapped with miRNAs that distinguished TAs from TVHG. As a result, we combined low-grade TAs with HPNMs to identify miRNAs specific to a "late," or more histologically advanced polyp. This approach yielded miR-145 and -619 that, when combined, showed accurate discrimination between the high-risk TVHG group and the lower-risk polyp group (HPNM + TA) without serrated histology. Specifically, a one-standard deviation decrease in miR-145 was associated with 18-fold increased odds of TVHG, whereas a one-standard increase in miR-619 was associated with a 7.7-fold increased odds of TVHG. Specifically, a one-standard deviation decrease in miR-145 was associated with 18-fold increased odds of TVHG, whereas a one-standard

deviation increase in miR-619 was associated with a 7.7-fold increased odds of TVHG. Inclusion of the expression of these two miRNAs yielded a c-index of 0.96 for segregating the HGTV high-risk from low-risk lesions (Supplementary Table S4).

Among those miRNAs differentially expressed between HPNMs and TVHGs, we found that miR-26a, 194, and -107 were overexpressed in the normal/low-risk tissues. Similar to miR-30a, miR-26a exhibits regulatory function in the cell cycle, acting as a putative anti-oncomiRNA. Although miR-26a has not been as widely described in the colorectal cancer, it has been reported to inhibit cell growth and tumorigenesis through repression of the EZH2 oncogene in nasopharyngeal carcinoma (32). In particular, miR-26a suppressed the expression of *c-myc*, the cyclins D3 and E3, and the cyclin-dependent kinases CDK4 and CDK6, while enhancing the expression of CDK inhibitors. miR-194 has been studied in colorectal cancer, where it was reported to be significantly suppressed in cancerous tissues (33). Furthermore, the authors noted that decreased levels of miR-194 expression was associated with tumor size, differentiation, and TNM stage (33). In a recent study, low expression of miR-194, adenoma size >2 cm, and >3 adenomas were shown to have independent risk factors for adenoma recurrence (34). In particular, it was found that in a 158-patient cohort, the sensitivity and specificity of miR-194 as a predictor were 71% and 78%, respectively, at a cutoff value of 0.13. Although we found this miRNA to differentiate high-grade adenomas from normal tissue, it was not found to be among the more accurate predictors to separate low- from high-risk polyps. Less studied is miR-107, although overexpression has been associated with greater sensitivity to 5-fluorouracil for colorectal cancer, through an unknown mechanism (35). This association may reflect underlying molecular heterogeneity among colorectal cancers that have previously been suggested to explain 5-FU sensitivity, including the debated relevance of microsatellite stability as a predictor of treatment responses (36).

Our observation of differentially increased miR-335 in non-serrated adenomas suggests that Wnt pathway activity may be a major discriminating factor between serrated and non-serrated polyp types. miR-335 has been previously shown to be upregulated in response to canonical Wnt signaling in human mesenchymal stem cells (37). Activation of canonical Wnt signaling is a common event in conventional adenomatogenesis, with loss of the adenomatous polyposis coli (APC) gene and translocation of β -catenin to the nucleus (38). Similarly, lower expression of miR-214 amongst non-serrated adenoma is consistent with a proposed inhibitory role on β -catenin pathway activation, where loss has been associated with invasion and stem-like traits in a number of cancers (39–41).

The finding that miR-125b and -199a were uniquely downregulated in TSAs relative to HPNMs is unique to this study. To our knowledge, no other study has assessed differences based on serrated histology. While miR-125b has previously been identified as downregulated in colorectal cancer, others reported it to be overexpressed. Interestingly, in the cancer patient series described by Bartley and colleagues (15), miR-125a, -125b, and 199a were identified as altered in high-grade dysplasia associated with invasive adenocarcinoma, but not low-grade foci. This result suggests that these changes may reflect underlying processes related to invasiveness. The miR-125 family includes three homo-

logs (-125a, -125b, and -125-2), with evidence for both oncogenic and tumor suppressive functions amongst members (42). Of interest is the evidence that miR-125 family members act as regulatory molecules in immune function, including potentiating the activity of macrophages for antigen presentation, killing of tumor cells (42), and maintenance of the immunosuppressive function of regulatory CD4⁺ T cells (Treg) that includes conferring protection against experimental colitis (43).

This is the first study to assess miRNA patterns in a cancer-free population undergoing screening with expertly adjudicated polyp histologic review. While our study is limited to 825 from up to 1800 miRNAs in current databases and not is comprehensive, our results demonstrate that specific miRNAs reproducibly shown to be altered in colorectal cancer show high accuracy to distinguish adenomas with low versus high malignant potential to the cancer-free population undergoing screening colonoscopy and expert histologic review. Given the challenges of managing the costs associated with benign adenoma detection during endoscopy-based screening and surveillance for colorectal cancer, these findings highlight the potential value of miRNA profiling in identifying patients with biologically high-risk adenoma diagnosed in busy community practices not benefitting from extensive histopathologic review by pathologists expert in adenoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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